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THERMAL INACTIVATION OF VIRUSES

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A review of the literature pertaining to thermal inactivation of virus in fluid media, fluid foods and solid foods indicated that the majority of viruses are inactivated at 71° C for 1 minute when normally expected levels of contamination occur. A limited number of viruses have been reported to require a higher heat process. Whether low level contamination by such viruses, would be expected in foods, would survive the 71° C level is not known because of the limited data available. Most food processes will not inactivate viral nucleic

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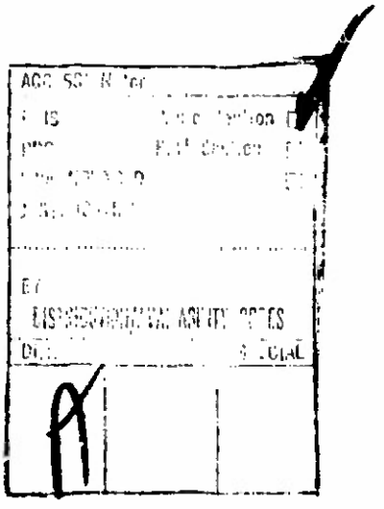
acids. However, the presence of nucleases, pH extremes in foods and long storage times probably reduce their potential for retaining infectivity.



PREFACE

The U. S. Army's food irradiation program has as its primary objective the use of high doses (>1 Mrad) of irradiation to make prepackaged enzyme inactivated meats stable and safe from a microbiological health hazard when stored under nonrefrigerated conditions. In this process (radappertization) foods are formulated, placed in cellulose casings or metal molds, heated to an internal temperature of 73 to 77°C to inactivate autolytic enzymes, and chilled to -3 to 5°C. The product is then vacuum packaged in cans or in flexible pouches, frozen to ca. -40°C, and irradiated within a temperature range of -40°C to -8°C to obtain the desired minimal radiation dose (MRD). Inoculated pack studies with 10 strains of C. botulinum spores provide data for the computation of the MRD, the dose required to reduce the number of viable spores of C. botulinum by a factor of 1×10^{12}.

Although viruses are more radiation resistant than C. botulinum spores, they should not present a health problem in radappertized meats because; due to their reported heat sensitivity, they should be destroyed during the preirradiation thermal treatment to inactivate autolytic enzymes. This review of literature, performed under project order number DRXNM 77-115 was deemed essential to confirm the reported heat sensitivity of viruses.

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THERMAL INACTIVATION OF VIRUSES

The thermal resistance of virus in foods is enhanced by the presence of protective agents that reduce the lethal effect of heat on the viruses at temperatures below 60° C. In addition, when solid foods are processed heat transfer is by conduction, and the required internal temperatures must be attained to effectively inactivate viral contaminants.

In Table 1 some of the characteristics of animal viruses are depicted. The various families have been separated by their buoyant densities and are listed alphabetically. The families 11 through 15 have been separated and also listed alphabetically. This same system was used to depict the information published in the literature pertaining to the familial resistance of viruses in laboratory media and in foods.

Examination of the data shown in Tables 2 and 3 indicate that the vast majority of viruses within the various families are inactivated at temperatures of about 60° C or less. This literature review on viral inactivation indicates that resistance to thermal denaturation appears to be restricted to viruses within the last five families depicted in Table 1. Greater resistance to temperature inactivation has been reported for the parvoviridae, the papoviridae and the picornaviridae. The adenoviridae and the reoviridae appear to be only slightly more resistant to thermal inactivation than the first ten families. Only the parvoviridae and the picornaviridae contain sufficient numbers of viruses to be of importance in studies on viral thermal inactivation. The greatest number of viruses of public health importance are in the family of picornaviridae. At the present time the exact position in the classification system of the hepatitis and gastroentertitis viruses is not known but probably they will be included in the parvoviridae or the picornaviridae.

The thermal inactivation data published in the literature have been concerned chiefly with inactivation of high titers of viruses in fluid preparations. There is great variation in the techniques and procedures used to evaluate the thermal resistance of viruses. In the majority of studies the viral suspensions were heated in test tubes that were held in constant temperature water baths. In some cases the viral suspensions were mixed in an attempt to equalize the temperature in the suspending media. In addition, a number of containers were used to process the virus suspensions such as. capillary tubes, ampules and varying types of flasks and bottles. It has been our experience that in evaluating the thermal resistance of viruses that even distribution of the heat occurs only when the viral suspension is processed in ampules or capillary tubes that are submerged in a constant temperature bath. When other types of non-submerged containers are utilized, there is uneven distribution of the heat and possible contamination of the heat processed fluids by viruses surviving on the walls and closures of the vessels. Such contamination may be interpreted as apparent survival of low level virus after the heat treatment process In some cases these viruses are not detected by cell culture systems but were observed when animal inoculation was utilized. It is possible that free virus nucleic acid may have been the causative agent in some of the reported virus persistence papers, especially when the heat treated preparation was injected into an animal.

In Table 4 a number of substances are listed that have a protective effect on viruses when low temperatures (40 to 60° C) are used in thermal inactivation studies. In addition, this protective effect has been observed with other substrates such as serum, ice cream mix and other high protein-carbohydrate containing suspensions. It has been shown that with higher temperatures, in excess of 60° C, the protective effect on the virus decreases significantly. When virus suspensions containing less than 10^4 particles/ml are processed, normal inactivation occurs in a short period of time at temperatures of 60 to 70° C and appear to be inactivated at relatively the same rate.

Only a limited number of viruses have been reported to have extremely high thermal resistance. These viruses are foot-and-mouth disease virus, hepatitis "A" (infectious hepatitis), hepatitis "B" (serum hepatitis) and the limited number of viruses in the papova and parvo virus families. In the reported foot-and-mouth disease studies a number of investigations were performed using high titers of viruses and in the majority of investigations tubes or bottles were used to process the suspension in the water bath. Apparent survival of low levels of viruses is possible with this procedure. Only limited or no viruses were reported to survive the heat processing of foods naturally contaminated by foot-and-mouth disease virus. Expected virus levels in naturally infected animals are shown in Table 5. Research in the Food and Drug Administration laboratories in Cincinnati indicates that virus levels of this magnitude were inactivated by temperatures less than those required for pasteurization of ice cream mix (65.3° C for 30 min or 79.4° C for 25 sec).

The reported thermal inactivation data on the hepatitis viruses are limited. In most cases viruses of unknown titer were heat-treated in blood or blood components in an attempt to inactivate viruses, and infectivity was determined by human or primate feeding or inoculation studies. In one series of studies using hepatitis "A" in marmoset serum a temperature of 60° C for one hour was not sufficient to prevent infectivity in marmosets injected with the heated product. When the same virus was suspended in water and heat processed, a reduction in infectivity was reported for the same time-temperature process. Because of the limitations in the reported data and the scarcity of information, the thermal resistance of hepatitis viruses is presently unknown.

It is possible that viral persistence may occur in heat processes using long time-temperature procedures such as cooking solid foods where periods of hours are required to reach internal temperatures of 60° C. Some viruses that could be present in animal foods are shown in Table 7. If viral protective agents are present in food, the lethality of the heat treatment process at levels below 60° C may have only a slight effect on the viruses, and the actual kill will commence only at temperatures above the 60° C. This could cause persistence of viruses in the food unless such considerations are made in determining the total food process, and the

lethality is calculated for the time-temperature process after reaching the 60° C level.

All the reported thermal inactivation data have been incorporated into Figure 1. Because of the variation in the inactivation rates of the different viruses, a range of inactivation data is shown. Normal virus concentrations expected to be encountered in food should be inactivated at the time-temperature points shown in the figure. Only limited virus inactivation data have been reported in solid foods and consideration of this deficit was given, as well as the questionable persistence of some viruses, when the data were used to plot the solid food slope. This slope is close to that of the USPHS Pasteurization Standard for ice cream mix.

Thermal inactivation of viruses is directly related to the chemical composition of the particle. The major organic constituents changed by temperature are lipids, proteins, and nucleic acids. The density of the particle is related to the varying compositions of the three organic constituents. Particles containing lipids or lipid complexes will tend to be more buoyant than particlea composed of carbohydrate, protein, and nucleic acids.

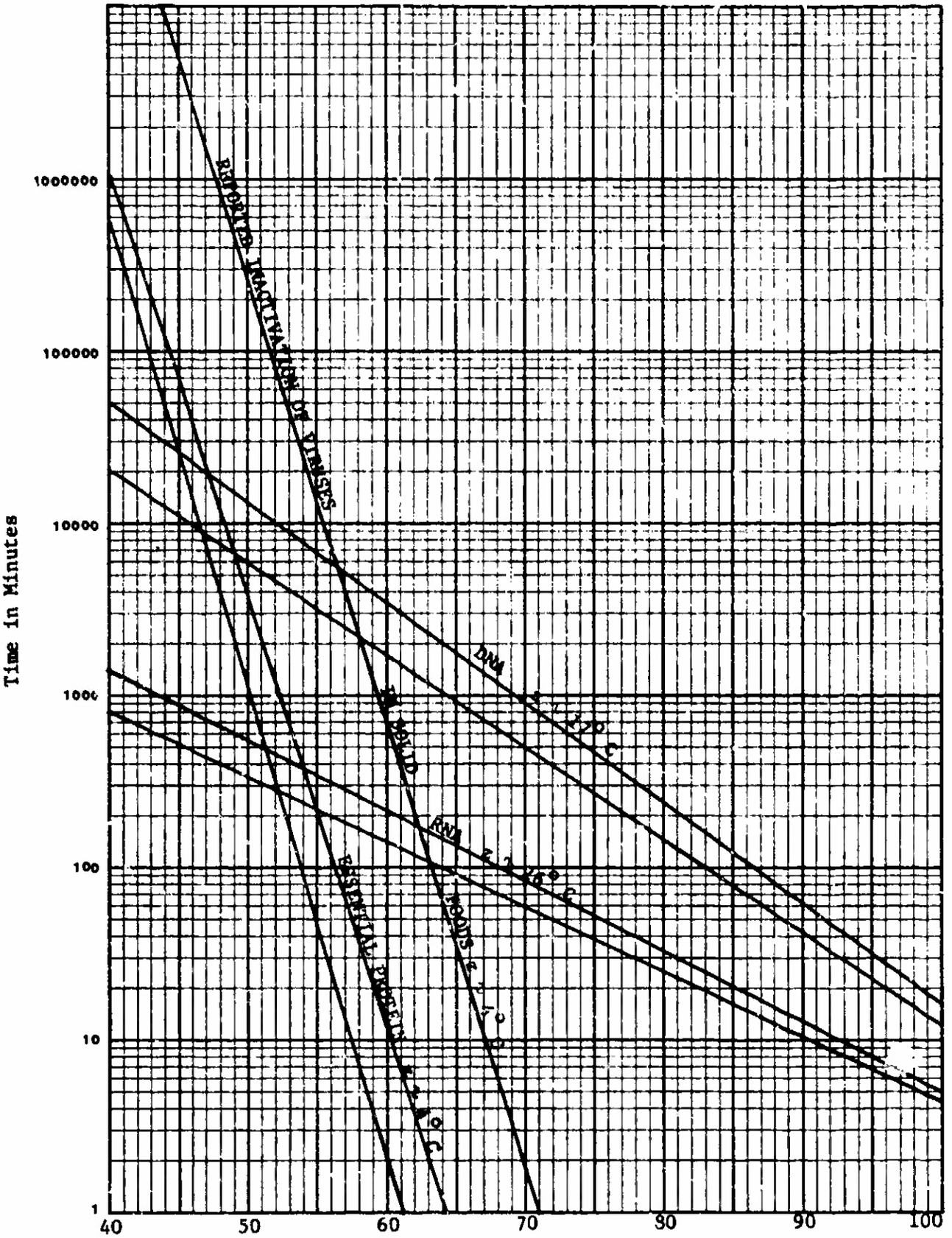
Low temperature (20° to 35° C) inactivation of viral infectivity is dependent upon disruption of the nucleic acids. At higher temperatures (> 50° C), inactivation is due to denaturation or disruption of the proteins of the virus particle.

The nucleic acids present in the virus are of two types: ribonucleic acid and deoxyribonucleic acid. In a minority of the cases the nucleic acid may be double stranded. The nucleic acid is highly resistant to thermal inactivation at high temperatures for short periods of time. However, the nucleic acid molecule is disrupted gradually at low temperatures over relatively long time spans.

Some of the thermal characteristics of nucleic acids are shown in Table 6. In the case of the RNA containing viruses, nucleic acid inactivation is probably due to breakage of the phosphate bonds, whereas, in the case of the DNA containing viruses, inactivation is due to cleavage of the purine or pyrimidine bases of the nucleic acid complex. At low temperatures, DNA is about 30-fold more resistant than the RNA. In Figure 1 inactivation of the RNA occurs in a period of 13-25 hours, whereas the DNA inactivation requires 13-35 days. Complete inactivation is dependent on the viral titer: the higher the nucleic acid content - the longer the period of time necessary for inactivation. At temperatures of approximately 30° - 45° C it is possible to inactivate nucleic acid with little apparent denaturation of the protein containing units of the virus particle. Little information is available pertaining to resistance of single stranded nucleic acid as compared to double stranded nucleic acid.

Figure 1

Time-Temperature Profile for Inactivation of 3 Logs of Virus Single Stranded Nucleic Acid or Essential Protein in a Fluid Medium



However, it appears that the complex structure of the double stranded DNA is more resistant to disruption than is single stranded DNA, and double-stranded RNA is more heat sensitive or equivalent to that of the single-stranded RNA.

Virus particles whose outer surface contain envelope structures are susceptible to mechanical injury. Such particles appear to be very sensitive to thermal inactivation. In many cases the envelope is composed of lipid complexes. Whether loss of infectivity is due to a mechanical injury of the envelope or to a change in protein or lipid structures is at present unknown.

Viruses with protein outer surfaces are thermally inactivated by denaturation of the protein that occurs at higher temperatures. In some cases the coat is ruptured and the nucleic acid is liberated into the medium. A certain percentage of particles entrap the nucleic acid in the core as the protein components on the surface are denatured. This particle has lost its ability to attach to the cell due to disruption of the structural integrity of the attachment site. However, these particles still containing nucleic acid may enter into the cell system by some mechanical process such as pinocytosis, or some other means of engulfment, and the nucleic acid may be liberated within the cell and infection occurs.

It has been demonstrated that the nucleic acid from a suspension of virus particles is liberated into the medium during a thermal inactivation process. If nucleases are present, the nucleic acids are rapidly inactivated. However, in the absence of nucleases, the liberated nucleic acid is infectious. Laboratory studies have shown that demonstrated infectivity by nucleic acid is 2 to 4 logs less sensitive than that of the intact viroid. Thus, 10^5 virus particles may be inactivated but infectivity is still demonstrated in the cell or animal system because of the low level infectivity of the free nucleic acid.

The thermal process used to inactivate enzymes in foods, not less than 73° C or more than 77° C, probably will be greater than that required to inactivate viruses. In Figure 1, a 3D virus inactivation process is depicted. If a 12D process is required, the slope will approach the USPHS pasteurization standard for ice cream mix when high temperatures are used. At low temperatures ($<<60^{\circ}$ C) longer time-temperature processes will be needed to inactivate the viruses than is required by the pasteurization standard.

Table 1. Some characteristics of animal viruses.

	Family	Denaity[a] (Cesium chloride)	Nucleic acid (Type-stranded)	Surface characteristics (Lipid)	(Envelope)
1.	Arenaviridae	1.18[1]	RNA, SS	+[b]	+
2.	Bunyaviridae	1.20-1.23	RNA, SS	-[c]	+
3.	Coronaviridae	1.19-1.23	RNA, SS	-	+
4.	Herpetoviridae	1.27-1.29	DNA, DS	+	+
5.	Orthomyxoviridae	1.17-1.20	RNA, SS	-	+
6.	Paramyxoviridae	1.21-1.24	RNA, SS	+	+
7.	Poxviridae		DNA, DS	+	-
8.	Retroviridae	1.16-1.18[1]	RNA, SS	+	+
9.	Rhabdoviridae	1.20	RNA, SS	-	+
10.	Togaviridae	1.25	RNA, SS	+	+
11.	Adenoviridae	1.33-1.35	DNA, DS	-	-
12.	Papovaviridae	1.34	DNA, DS	-	-
13.	Parvoviridae	1.38-1.46	DNA, SS	-	-
14.	Picornaviridae	1.32-1.41	RNA, SS	-	-
15.	Reoviridae	1.31-1.38	RNA, DS	-	-

[a] g/cm^3; [b] + present; [c] - abaent

(1) In sucroae gradient

Abbreviationa: RNA-ribonucleic acid, DNA-deoxyribonucleic acid, SS-single stranded and DS-double stranded.

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Some Characteristics of Animal Viruses

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Table 2. Virus thermal inactivation.

	Family	Virus	Titer	Thermal Effect Temp. °C	Thermal Effect Time min.	Remarks	Method of inactivation*	Reference
1.	Arenaviridae	Lymphocytic choriomeningitis				Extremely thermolabile		Andrewes
		American haemorrhagic fever viruses				Readily inactivated		
2.	Bunyaviridae					Thermal characteristics similar to Togaviridae		Andrewes
3.	Coronaviridae	Mouse hepatitis		56	30	Inactivated		Andrewes
		Infectious bronchitis (birds)		56	30	Inactivated		
		Transmissible gastro-enteritis (pigs)		56	30	Inactivated		
		Haemagglutinating encephalomyelitis		56	30	Inactivated		
4.	Herpetoviridae	Herpes simplex	$\sim 10^5$	50	20	$\sim$ 5 log loss in infectivity in tris buffer		Plummer
		Cytomegalovirus	$\sim 10^4$	50	40	$\sim$ 2.5 log loss in infectivity in tris buffer		
		Cytomegalovirus	$\sim 10^6$	44	19.9 half life	Tissue culture fluid and 2% calf serum	WB, T	Krugman
		Laryngotracheitis		37	> > 3 yrs	Persisted, lyophilized allentoic fluid		Hofstad
		Herpes		50 - 52	30	Inactivated		Andrewes
		Cytomegalovirus		50	40	"		Dupré
				56	30	"		
		Herpes simplex		52	30	"		

*WB-water bath
T-test tubes
a-ampules

Table 2. Virus thermal inactivation. (contd.)

	Family	Virus	Titer	Thermal Effect Temp. °C	Thermal Effect Time min.	Remarks	Method of inactivation*	Reference
5	Orthomyxoviridae	NM strains	10^5 to 10^8	62	30	Virus survived, mouse brain emulsions or infected fluids		Burnet
		Influenza A		61		Inactivated		Francis
		Influenza B		56	15-30	Inactivated, allentoic fluid		
		Influenza A2/ Aichi/2/68		56	22	Four log loss, allentoic fluid		De Flora
		Influenza A PR-8		56	25	Inactivated, phosphate buffered saline		Siegert
		Influenza A	$\sim 10^4$ to 10^5	45	10	Inactivated, allentoic fluids	WB-T	Lauffer
		Orthomyxoviruses		50	30	Usually inactivated		Andrewes
6	Paramyxoviridae	Respiratory cyncytial	10^3 to $10^{6.9}$	50	mins	Pellet suspended in distilled water or sodium chloride solution (0.3 M) < 3 log drop in infectivity	WB-T	Rechsteiner
		Newcastle disease		37	~ 10 mos.	Inactivated, lyophilized allentoic fluid		Hofstad
		Newcastle disease	10^{12}	79-81	0.033 sec.	Inactivated, allentoic fluid	HT-ST tubular apparatus	Dutcher
		Measles	$> 10^4$	56	30	Inactivated, tissue culture fluid		Black
		Paramyxoviruses				Heat labile		Andrewes
		Measles		56	25	Inactivated, buffered saline		Dupré
		Mumps		55	20			
		Mumps		56	20			

Table 2. Virus thermal inactivation. (contd.)

	Family	Virus	Titer	Thermal Effect Temp. °C	Thermal Effect Time min.	Remarks	Method of inactivation*	Reference
7	Poxviridae	Fowl pox		37	> 2 yrs.	Persisted, lyophilized allentoic fluid		Hofstad
		Variola		55	Half life 2 minutes	In saline	WB, a	Hahon
		Vaccinia	10^8	60	10	> 7 log loss in infectivity, last fraction persisted for more than 60 min, citrate buffer	WB, T	Kaplan
		Vaccinia	$\sim 10^6$	56	30	~ 5 log loss in infectivity		Sharp
		Vaccinia	5×10^8	56		~ 6 log drop in titer		Galasso
				60		Inactivated, tissue culture medium		
		Goat poxvirus	$\sim 10^5$	50	60	Extracted from scabs,		Pandey
		Sheep poxvirus	$\sim 10^5$	50	60	~ 4 log drop in infectivity of both viruses		
8	Retroviridae	Avian erythroblastosis	High	56	6	99% drop in infectivity, plasma	WB, T	Bonar
			High	37	minutes	In plasma, rapid	WB, T	Eckert
				56	seconds	Inactivation, tailing		
		Rous sarcoma	High	60	6	Phosphate buffer saline + 2% horse serum - half life at 60° is 0.7 min		Dougherty
		Moloney	$\sim 10^3$	56		Inactivated, sodium citrate buffer	WB, T	Moloney
		Murine sarcoma (Moloney)	$\sim 10^4$	60	~ 6.5	Inactivated, tissue culture fluid	WB, T	Nakata
		AK	$\sim 10^3$	50-55	30	Inactivated		Sinkovics
		Graffi	$\sim 10^3$	56-65	30	Inactivated		

Table 2. Virus thermal inactivation. (contd.)

Family	Virus	Titer	Thermal Effect Temp. °C	Thermal Effect Time min.	Remarks	Method of inactivation*	Reference
8 Retroviridae (contd.)							
	Friend	$\sim 10^{3-4}$	56	30	Inactivated		
	Moloney leukemia	$\sim 10^{3-4}$	56	30	Inactivated		
	Mouse lymphocytic leukemia	$\sim 10^{3}$	56	30	Inactivated, cell free filtrate		Buffet
	Rauscher leukemia	$\sim 10^{4}$	56	30	Inactivated, sodium citrate buffer	WB, T	Geigel
	Bovine leukemia		60	30 sec.	Inactivated, in tissue culture fluid	WB, T	Baumgartene
9 Rhabdoviridae	Rabies Rhabdoviruses		60	5	Inactivated. Relatively heat sensitive		Schultz Andrewes
10 Togaviridae	EEE	$10^{7.2}$	37	30 hrs	Tissue culture fluid, inactivated	WB, T	Nir
	WEE	$10^{5.6}$	37	48 hrs			
	Sindbis	$10^{5.6}$	37	36 hrs			
	SLE	$10^{6.0}$	37	48 hrs			
	West Nile	$10^{5.6}$	37	72 hrs			
	Ntaya	$10^{4.0}$	37	30 hrs			
	Semliki Forest		50	20	> 2 log loss in infectivity	WB, T	Fleming
	Modoc	$10^{6.0}$	37	72 hrs	~ 2.5 log loss, phosphate buffer saline + 0.5% calf serum		Davis
	Japanese B encephalitis	$10^{6.0}$	37	90 hrs	> 6 log loss in infectivity, tissue culture fluid	WB, a	Darwish

Table 2. Virus thermal inactivation (contd.)

Family	Virus	Titer	Thermal Effect Temp. °C	Thermal Effect Time min.	Remarks	Method of inactivation*	Reference
10 Togaviridae (contd.)							
	Tickborne encephalitis	10^7 to 10^9	50	12	Tissue culture fluid, inactivated	WB, T	Mayer
	WEE	$10^{7.6}$	56	30	Extract from mouse brain, ∿ 4 log drop		Fastier
	EEE	∿ 10^7	50	> 4 hrs	Survived, ∿ log drop	WB, a	Mika
	VEE	∿ 10^7	50	> 7 hrs	PBS, survived, ∿ 5 log drop		
	EEV	10^8	56	2 hrs	Inactivated		Mahdy
11 Adenoviridae	Adenovirus		56	30	Inactivated		Andrewes
	Adenovirus	> 10^2	56	30	Inactivated		Huebner
	Infectious bronchitis		37	6 mo	Inactivated, lyophilized allentoic fluid		Hofstad
	Ovine adenovirus		60	30	Inactivated		Andrewes
	Adenoviruses		56	2.5-5	Inactivated		Dupre'
12 Papovaviridae	S. E. polyoma		70	30	Tissue culture fluid survived, inactivated	WB, a	Eddy
			80	30			
	Mouse polyoma		60	30	Tissue culture fluids no effect on infectivity	WB-vials, screw cap	Brodsky
			65	30	4 log drop in infectivity		
			70	30	> 6 log drop in infectivity		
	Rabbit papilloma		70	30	Inactivated		Andrewes
			67	30	Survived		
	Oral papillomatosis		65	30	Survived		

Table 2. Virus thermal inactivation (contd.)

Family	Virus	Titer	Thermal Effect Temp. °C	Thermal Effect Time min.	Remarks	Method of inactivation*	Reference
12 Papovaviridae (contd.)							
	Human wart		50	30	Survived		Andrewes
	Canine oral papillomatosis		58	60	Inactivated		Andrewes
	Mouse polyoma		70	30	Usually inactivated	WB, T	Andrewes
	K		70	4.5 hrs	Inactivated		Andrewes
			70	3 hrs	Survived		
13 Parvoviridae	Feline panleucopenia of cats		50		Fairly stable		Andrewes
			80-85	30	Inactivated		Andrewes
	Porcine parvo		70	2 hrs	Survived		Andrewes
	Adeno-associated		60	30 half life			Andrewes
14 Picornaviridae	SV-2 enterovirus		45	120 sec	Tissue culture fluid, rapidly inactivated	Screw cap vial, WB	Heberling
	Foot-and-mouth disease (FMDV)	10^7	55	60	∿ 6 log inactivation, resistant fraction, tissue culture fluids	WB, T	Bachrach(60)
	FMDV	∿ 10^7	61	3 sec	∿ 5 log reduction tissue culture fluids, diluted in veronal-acetate buffer	WB, a	Bachrach(57)
	FMDV		85	6 hrs	∿ 10^7 log - inactivated		Dimopoullos
	Coxsackie A-21		50	30	> 4.2 logs	loss	Dimmock
	Poliovirus 1		50	30	> 6.0 logs		
	ECHO-4		50	30	> 3.0 logs		
	ECHO-11		50	60	> 3.7 logs	in	
	Rhinoviruses HGP		50	60	> 2.8 logs		
	B.632		50	30	> 2.4 logs		
	FEB		50	60	> 2.5 logs	infectivity	

Table 2. Virus thermal inactivation. (contd.)

Family	Virus	Titer	Thermal Effect Temp. °C	Thermal Effect Time min.	Remarks	Method of inactivation*	Reference
14 Picornaviridae (contd.)							
	No.		50	60	3.6 logs loss		
	DC		50	60	> 3.3 logs		
	1098		50	60	2.7 logs in		
	C.V. 11		50	60	2.7 logs		
	S.D. 1		50	60	> 3.9 logs infectivity		
	Duck hepatitis	10^2 to 10^6	56	30	2 to 5 log loss in infectivity	WB, T	Hwang
	Poliovirus		60	30	Inactivated		Andrewes
	ECHO		65	30	Inactivated		
	Coxsackie A		55	30	Inactivated		Dupré
			53-55	30	Inactivated		
	Murine polio		60	30	Water, inactivated	WB sealed tubes	Lawson
	SK	10^3					
	Modified Lansing	1300/ml	55	30	Water, inactivated		
	TO_4	400/ml	65	30	Water, inactivated		
	Coxsackievirus		60	30	Water, inactivated		Melnick
	Coxsackievirus		55	30	Brain suspension, inactivated		Dalldorf
	Poliovirus 1		50		Most sensitive to heat		Papaevangelou
	2		50		Least sensitive to heat		
	3		50		Intermediately sensitive to heat		

Table 2. Virus thermal inactivation. (contd.)

Family	Virus	Titer	Thermal Effect: Temp. °C	Thermal Effect: Time min.	Remarks	Method of inactivation*	Reference
14 Picornaviridae (contd.)							
	Poliovirus		44-55	30	Inactivated		Schultz
	Poliovirus		55	5	Inactivated, extracts of cord and brain		Shaughnessy
			52.5	30	Inactivated		
		Low level	42.5	30	Inactivated		
	Poliovirus 2	$\sim 10^5$	56	30	Inactivated	WB, capillary tubes	Stanley
	Poliovirus	$10^{6.5} - 10^{7.3}$	75	60	Few survivors	WB, a	Medearis
	Poliovirus 1-2	$> 10^8$	50	24 hrs	$\sim$ 8 log drop	WB, vials, stoppers	Youngner
	Poliovirus 3	$> 10^6$	50	24 hrs	$\sim$ 6 log drop		
15 Reoviridae	Reovirus 3		56	Half life of 1.6 min	Tissue culture fluid + 5% calf serum	WB, T	Gomatos
	Reovirus		55	15	Inactivated		Dupre'
	African horse sickness		60	60	Persisted		Andrewes
Other	Hepatitis B		98	1	1/10 dilution of serum, inactivated	WB, T	Krugman
	Hepatitis B		60	10 hrs	Inactivated, serum	WB	Soulier
	Hepatitis A		100	5	Inactivated, water	WB, large, a	Provost
			60	60	Titer reduced, water		
			60	60	In serum, no apparent reduction in titer		
	Hepatitis A		60	60	Inactivated		Dupre'

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Table 3. Thermal inactivation of viruses in foods.

Family	Virus	Titer	Thermal Effect Temp. °C	Thermal Effect Time min.	Remarks	Method of inactivation	Reference
4 Herpeto-viridae	Herpes simplex	$\sim 10^4$	65	2 sec	Inactivated in ice cream mix	WB, a	Sullivan
6 Paramyxo-viridae	Newcastle disease (NCDV)	1(	64.4	160 sec	Suspended in whole egg ~ 3 log drop in infectivity in 160 sec. Survivors after 200 sec.	WB	Gough
	NCDV	Allentoic fluid diluted 1/100	60	5-7	Dependent on virus titer, inactivated	WB, a	Foster
8 Retro-viridae	Rauscher leukemia	$\sim 10^3$	50	5	Inactivated,	WB, a	Sullivan
			55	5 sec.	ice		
	Moloney sarcoma	$\sim 10^3$	55	15	cream		
			55	15 sec.	mix		
	Rous sarcoma	$\sim 10^5$	55	25	Inactivated,		
			65	3 sec.	in ice cream mix		
	Bovine leukemia		73.5	42 sec.	Inactivated, in milk	Stainless steel tank	Baumgartener
10 Toga-	Swine fever	$10^{4.7} - 10^{6.0}$	65	30	Inactivated in ham	Canned	Terpstra
	Rift Valley fever		56	40	Inactivated, in blood		Dupre'

WB-Water bath
T-Test tubes
a-Ampules
C-Capillary tubes

Table 3. Thermal inactivation of viruses in foods. (contd.)

Family	Virus	Titer	Thermal Effect: Temp. °C	Thermal Effect: Time min.	Remarks	Method of inactivation*	Reference
10 Togaviridae (contd.)							
	Tickborne encephalitis	$10^{5.7}$	70	20	Inactivated, in milk	WB, a	Gresikova
			55	20	5 log loss, in serum		
			62	20	Inactivated, in serum		
		$10^{3.7}$	72	10 sec.	Inactivated, in milk	Laboratory pasteurizer	Gresikova
11 Adenoviridae	Adenovirus 14	$\sim 10^{4}$	65	2 sec.	Inactivated, in ice cream mix	WB, a	Sullivan
14 Picornaviridae	Swine vesicular disease	$\sim 10^{6.5}$	56	30	Inactivated, in milk		Herniman
			60	2			
			60	10	Inactivated, in fecal slurry		
			64	2			
	Murine poliomyelitis		70	30	Inactivated, in milk	WB, a	Lawson
	Poliovirus 1		75-80	4	Inactivated, in meat balls fried in oil		Aizen
	Coxsackievirus B-3		80-85	4			
	Poliovirus	2.5×10^{7}	60	30 sec.	> 6 log inactivation, in milk	Cheese process	Cliver
	Poliovirus 1	$\sim 10^{4}$			$\sim$ 2 log	Stewed oysters	Di Girolamo
				10	$\sim$ 2.5 drop	Fried oysters	
				20	$\sim$ 2.5 in	Baked oysters, oven	
				30	$\sim$ 2 virus	Steamed oysters	

Table 3. Thermal inactivation of viruses in foods. (contd.)

	Family	Virus	Titer	Thermal Effect Temp. °C	Thermal Effect Time min.	Remarks	Method of inactivation	Reference
14	Picorna-viridae (contd.)							
		Poliovirus 1	10^5 - 10^8	80	5	Survived, 47% fat most protective ground beef	WB, T, screw cap	Filppi
		Coxsackievirus A-9	7.5 x 10^5	49	6 hr	In Thuringer sausage ∿ 3 log drop in virus		Herrman
		Poliovirus 1	1 x 10^8	60	30	In sausage, internal		Kantor
		Echovirus 6	6 x 10^7	60	30	temperature, ∿ 1 log drop		
		Poliovirus	$10^{2.5}$ x $10^{3.5}$	61.7	30	Inactivated, in milk, cream and ice cream 1 sample in stainless steel tubes indicated survivors	WB, a, C and stainless steel tubes	Kaplan(52)
		Poliovirus Lansing	$10^{2.3}$ x $10^{4.0}$	79.5	15 sec.	Survived in ice cream	WB, C	Kaplan(54)
		MEF-1		82.2	25 sec.	(10% ground brain)		
		Y-S-K						
		Coxsackievirus	$10^{7.5}$	61.7	15	Inactivated,		Kaplan(54)
				71.6	30 sec.			
				72.1	30	in		
				67.7	30			
				71.1	15 sec.	milk		
		Coxsackievirus A-9		85	20 sec.	Inactivated, in milk		Kostenko
				75	15 sec.	Survivors, in milk		

Table 3. Thermal inactivation of viruses in foods. (contd.)

Family	Virus	Titer	Thermal Effect Temp. °C	Thermal Effect Time min.	Remarks	Method of inactivation*	Reference
14 Picorna-viridae (contd.)							
	Poliovirus 1	3×10^5	71		Inactivated, in beef patties	Broiled	Sullivan
	Coxsackievirus B-2	$1\text{-}3 \times 10^4$	71				
	FMDV	$10^{6.7} - 10^{7.5}$	65	64.5	Survived, in milk	WB, tubes	Hyde
			72	15-17 sec.			
			80	15-17 sec.			
			65		Survived, evaporation to 50% of liquid volume		
	FMDV	Natural contamination	90		Inactivated, milk		Felkai
			90	35 sec.	Inactivated, dried virus		
			80	70 sec.	In milk		
	FMDV	$1 \times 10^{7.8}$	80	6 hrs.	Inactivated, tissue suspension		Demopoullos
			80	4 hrs.	Not inactivated, tissue suspension		
	FMDV	$\sim 1 \times 10^6$	72	0.25	Survived, pelleted cell debris	WB, T	Blackwell
			72	5	Survived, whole milk		
			72	3	Survived, evaporated milk		
			93	0.25	Survived, cream		
	FMDV				Cheddar cheese and Camenbert cheese process, survived Inactivated in Mozzerella cheese process		Blackwell
			63	6 sec.	Survived in milk		

Table 3. Thermal inactivation of viruses in foods. (contd.)

Family	Virus	Titer	Thermal Effect: Temp. °C	Thermal Effect: Time min.	Remarks	Method of inactivation*	Reference
14 Picorna-viridae (contd.)							
	FMDV		55	20	Inactivated, in blood		Dupre
			60	5	Inactivated, in vesicular fluid		
			85	360	Cattle tongue suspension		
	Porcine enterovirus F-7		65	2	Inactivated		Kelly
	Echovirus 6 and Poliovirus 1	9.3×10^3	56.7	3.5	Inactivated, in egg white		Strock
		9.3×10^6	57.8	2			
			58.9	1.1			
	Echovirus 6	2.4×10^4	56.7	20	Inactivated, in egg yolk		
	Poliovirus 1	2.4×10^6	57.8	11			
			58.9	7			
			60.0	3			
	Foot-and-mouth disease	$\sim 10^{4.8}$			Boiling water bath 65 min, cooled in 45 min, peak internal temperature 74° C, inactivated	In cans	Heidelbaugh

Table 3. Thermal inactivation of viruses in foods. (contd.)

Family	Virus	Titer	Thermal Effect: Temp. °C	Thermal Effect: Time min.	Remarks	Method of inactivation*	Reference
14 Picornaviridae (contd.)							
	FMDV	$10^{4.5}$ - $10^{7.1}$	56	6	∿ 5 logs inactivated, pH 6.7	In bottles	Sellers
			63	1			
			72	17 sec.			
			80-85	> 5 sec.			
			56	30	∿ 5 logs inactivated, pH 7.6		
			63	2			
			72	55 sec.			
			80-85	> 5 sec.			
	FMDV		65	30 sec.	Inactivated in milk, skim milk, cream and powdered milk	WB, T	Moosbregger
	FMDV		65	30 min	Inactivated, in milk		Hastli
15 Reoviridae	Reovirus 1	∿ 10^{4}	65	12 sec.	Inactivated, ice cream mix	WB, a	Sullivan

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Table 4. Agents modifying virus thermal inactivation.

Virus	Remarks	Reference
Poliovirus	Mg^{++} stabilized virus at 45° C	Ackerman
Poliovirus 1	Cations stabilized virus at 45° C	Fujioka
Polioviruses 1, 2, 3	$MgCl_2$ stabilized virus during storage at 4°C	Melnick
Polioviruses 1 + 2 Echo 6 and 7 Coxsackie B-5	Reduced sulfhydryl groups Stabilized viruses to inactivation At less than 50° C	Halsted
Poliovirus 1	L-cystine stabilized, 5 different stocks of virus to temperatures below 50° C	Pohjanpelto
Poliovirus 1	Elemental sulfides (tetrasulfide) Stabilized virus against inactivation at 50° C	Pons
Poliovirus 1, 2, 9, 3 Coxsackie A-9, B-3 and Echo-1 Echo-3, 6 and 19	50 µg/ml of L-cystine stabilized viruses 500-2500 µg/ml stabilized of the viruses No stabilization	Pohjanpelto
Echo-32	$MgCl_2$ stabilized virus at 50° C	Branche
Poliovirus 2	0.1 M NaCl at 56° C for 1 hr ∿ 6 log drop in infectivity 2.0 M NaCl at 56° C for 1 hr ∿ 2 log drop in infectivity	Speir
Rhinoviruses and Enteroviruses	$MgCl_2$ less effectively stsbilized Rhinoviruses at 50° C	Dimmock
Simian virus SV-2	$MgCl_2$ stabilized virus at 50° C	Heberling
Herpesvirus (JES)	Na_2SO_4 and Na_2HPO_4 stabilized at 50° C $MgCl_2$, $MgSO_4$, KH_2PO_4, 2 MKCl or NaCl did not stabilize virus Very thermosensitive in isotonic salt solution	Wallis

Table 4. Agents modifying virus thermal inactivation (contd.)

Virus	Remarks	Reference
Rhabdoviruses	EDTA and serum demonstrated protective effect on virus at 37 and 56° C	Michalski
Newcastle disease	Casein protected virus at 45° C $MgSO_4$ did not protect virus	Ballesteros
Tick-borne encephalitis	Monovalent metallic cations stabilized virus at 50° C	Mayer
DNA viruses	Not stabilized by divalent cations	Wallis
Reovirus	Stabilized by divalent cations	Wallis
Vesicular exantchoma of swine	No cationic stabilization at 50° C characteristic of human enteroviruses only	Zee

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Table 5. Naturally occurring virus concentrations in Food Animal Products

Virus	Titer	Remarks	Reference
Foot-and-mouth	10^4	In serum, bovine	Burrows
Disease virus	$10^{4.5-5.3}$	Pharynx	
(FMDV)	$10^{3.3-5.2}$	Milk	
	10^3	Saliva	
FMDV	$10^{4.5}$	Bone marrow, bovine	Cox
FMDV	$10^{2.0}$	Muscle, brain	Cottral
	$10^{4.0-5.0}$	Blood	
FMDV	$10^{0.7}$ to $10^{2.1}$	Beef, bovine	Henderson
	$10^{1.5}$ to $10^{2.2}$	Liver	
	$10^{1.7}$	Kidney	
	$10^{2.4}$	Blood	
	$10^{0.7}$ to $10^{2.8}$	Rumen	
	$10^{1.8}$ to $10^{2.3}$	Lymph node	
Tickborne encephalitis	$\sim 10^{3.7}$	Goat milk	Gresikova
Enteroviruses	$< 10^1$	Shellfish	Denis
Swine fever virus	$10^{4.7}$ to $10^{6.0}$	Blood	Terpstra

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Table 6. The effect of temperature on nucleic acids.

Virus	Remarks	Reference
Foot-and-mouth disease (FMDV)	Virus heated to 61° and 85° C in RNase free medium produced infectious nucleic equivalent to that produced by phenol extraction	Bachrach
FMDV	Prolonged incubation at 25° C for 24 hrs or 37° C for 8 hrs resulted in a loss of ∿ 3 logs of infectious nucleic acid	Brown
Tobacco mosaic virus (TMV)	Loss of RNA biological activity occurs at moderate heat in marked contrast to DNA which is inactivated with a high-temperature coefficient	Ginoza
Poliovirus 1 and TMV	∿ 90% loss of RNA infectivity in 80-120 min at 65° C ∿ 99% loss of RNA infectivity in 40-45 min at 80° C ∿ 99% loss of RNA infectivity in 3-5 min at 100° C	Gordon
Poliovirus 1	Virus rapidly degraded st 56° C liberating viral RNA	Jordan
Polioviruses	Protein densturation precedes dissociation of viral RNA at 50° C	Meltens
EEE and VEE	RNA inactivated ∿ 90% in 4 hrs st 50° C	Mika
Poliovirus	Heating RNA for 5 min at 60° C results in loss of 1 log of infectivity - virus heated same procedure results in 7 log loss of infectivity	Norman
Poliovirus mutants	Variation in sensitivity to heat mutants	Papaevangelou
Bacteriophage	Heat (50-60° C) inactivation is accompanied by release of native DNA. The molecules are intact - 90% removed from virus in 60 min at 60° C	Ritchie

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Table 7. Viruses producing systemic infections in food animals (North America)

Virus group	Type of Animal: Bovine	Porcine	Avian
RNA Viruses:			
Picornaviruses	Enteroviruses (+?)[a] Encephalomyocarditis virus +[b]	Enteroviruses (+?)	Enteroviruses (+?)
Myxoviruses	Parainfluenza 3+	Swine influenza (type A)+	Influenza virus (type A)+ Newcastle disease virus+
Reoviruses	Types 1, 2, and 3+	Probably types 1, 2, and 3+	Probably types 1, 2 and 3+
Togaviruses	Encephalitis viruses+		Eastern encephalitis+ Western encephalitis+
Retroviruses	Bovine leukemia virus		Rous sarcoma+, Avian Leukosis complex
Other RNA viruses	Bovine diarrhea virus BVD mucosal disease Vesicular stomatitis virus+ Rabies virus+ Bovine syncytial virus (?)[c]	Hog cholera virus Vesicular stomatitis virus+ Lymphocytic choriomeningitis+	Infectious bronchitis virus
DNA Viruses:			
Herpes viruses	Pseudorabies virus (+?) Malignant catarrhal fever virus	Pseudorabies virus (+?)	Marek's disease virus Herpes turkey virus
Adenoviruses	3 serotypes (?)	At least 3 serotypes (?)	At least 10 serotypes (?)
Other DNA viruses		Hemagglutinating encephalomyelitis	Avian encephalomyelitis, viral arthritis agent

[a] (+?) Systemic infection and infectious for man possible.

[b] + Infectious for man.

[c] ? Systemic infection probable.

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